

## RESISTANCE TO INSECTICIDES IN THE LARVAE OF *CULEX FATIGANS* IN MALAYA

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### SYNOPSIS

After two years' use of hexachlorocyclohexane (BHC) as a larvicide in Georgetown, on Penang Island, control of *Culex fatigans* breeding became unsatisfactory. Two laboratory colonies of *fatigans* were established, one from Georgetown, and one from Kuala Lumpur where no insecticides had been used; tests were then made to determine the median lethal concentrations (MLC) of BHC, dieldrin, and DDT for the larvae of the two strains. The Georgetown strain was found to have acquired a tenfold resistance to BHC, and also to dieldrin to which it had not been exposed, but it showed no significant increase of resistance to DDT, to which it had also not been exposed. A year later, when both strains had passed through some ten generations in the laboratory without exposure to insecticides, the Georgetown strain was found to have lost much of its resistance to BHC, although the MLC was still twice that of the non-resistant Kuala Lumpur strain.

In 1950, after field trials made by Byrne<sup>2</sup> at the beginning of that year, the Municipal Health Department of Georgetown (population about 190,000, area 9.5 square miles (approximately 24 km<sup>2</sup>), on Penang Island, substituted hexachlorocyclohexane (BHC) wettable powder for oil in the routine weekly larviciding programme. The BHC (Gammexane P.520) was used at the rate of 3 ounces of powder per Imperial gallon (18.7 g per litre) and was applied in the usual way with knapsack sprayers, giving a dose of probably about 4 ounces of the gamma-isomer per acre (about 280 g per hectare). Larval control was good, and was said to be still satisfactory some two years later at the end of 1952. Early in 1953, however, the staff reported that larvae of *Culex fatigans* were still present in considerable numbers the day after spraying, and by mid-year double the previous dose of BHC did not give satisfactory control.

### Material and Methods

The author visited Georgetown at the end of June 1953 and, with the help of the Municipal Health Department staff, collected some egg

rafts of *C. fatigans* from one of the drains where control had conspicuously failed. These rafts were brought back to Kuala Lumpur and a laboratory colony of *C. fatigans* was established from them (strain Penang G). At the same time a colony (Kuala Lumpur strain) was established from rafts collected in Kuala Lumpur where no BHC had been used. Tests with larvae were then commenced, to compare the relative susceptibility of the two strains to BHC, and also to DDT and dieldrin. At a later date a second strain of *fatigans* (Penang B.P. strain) was obtained from Penang; this came from Balik Pulau, which lies over the hills on the opposite side of the island from Georgetown, where no BHC had been used.

The methods employed were those described by Wharton.<sup>15</sup> Briefly, three-day-old larvae (early fourth instar) were exposed for 20 hours in glass beakers containing various strengths of acetone suspensions of the insecticides in water, and the dead and moribund larvae were counted at the end of that time. The results were analysed by the probit method and the median lethal concentration (MLC) was estimated visually from graphs.

### Results

The results are summarized in table I.

It will be seen from the first two lines in the right-hand column of the table that under the conditions of these tests the concentration of gamma-BHC required to kill 50% of the Penang G strain was 10 times greater than for the Kuala Lumpur strain—MLC 0.257 parts per million (p.p.m.) as compared with 0.026. In order to be sure that this was an acquired resistance and was not due to geographical differences between the *C. fatigans* of Kuala Lumpur and that of Penang, which are about 190 miles (306 km) apart, a strain of *fatigans* was obtained from another part of Penang where no BHC had been used (Penang B.P. strain). It will be seen that the MLC for this strain was actually lower than that for the Kuala Lumpur strain—0.017 p.p.m. as compared with 0.026.

The Penang G and Kuala Lumpur strains were then tested against dieldrin and DDT, to which neither had been exposed. The mortalities of the Penang G larvae exposed to dieldrin were rather irregular, but the estimated MLC was 10 times that for the Kuala Lumpur strain—0.060 p.p.m., as compared with 0.006. Evidently the tenfold resistance acquired to BHC had conferred a similar resistance to dieldrin. There was very little difference between the two strains with respect to DDT: Penang G, MLC 0.245; Kuala Lumpur 0.224. These results are in conformity with other work (Goodwin-Bailey & Davies;<sup>4</sup> Wilson, Gahan & McDuffie<sup>16</sup>) which has shown that there tends to be cross-resistance between BHC and dieldrin, but that little cross-resistance exists between these and DDT.

TABLE 1. LARVAE OF CULEX FATIGANS : PERCENTAGE DEAD AMONG DIFFERENT STRAINS AFTER EXPOSURE FOR 20 HOURS TO VARIOUS CONCENTRATIONS OF INSECTICIDE-ACETONE SUSPENSIONS IN WATER, AND THE ESTIMATED MEDIAN LETHAL CONCENTRATION

Insecticide	Mosquito strain	Number of replicates (25 larvae)	Concentration of insecticide (p.p.m.)										Median lethal concentration
			0.0025	0.005	0.01	0.025	0.05	0.1	0.25	0.5	1.0	2.5	
Gamma BHC	1953 Penang G Kuala Lumpur Penang B.P.	2-4	—	—	—	0	7	14	21	67	92	100	0.257
		3-5	—	0	2	9	85	100	—	—	—	—	0.026
		4-6	—	6	33	31	98	100	—	—	—	—	0.017
	1954 Penang G Kuala Lumpur	2-4	—	—	2	9	57	91	99	100	—	—	0.046
		1-4	—	—	12	21	96	100	—	—	—	—	0.024
Dieldrin	1953 Penang G Kuala Lumpur	2-5	0	3	28	18	27	24	75	—	—	—	0.060
		2-4	5	41	85	100	—	—	—	—	—	—	0.006
DDT	1953 Penang G Kuala Lumpur	2-4	—	—	—	—	0	6	55	90	—	—	0.245
		2-4	—	—	—	—	0	24	62	89	—	—	0.224

About a year after the completion of these tests, the question was raised whether the acquired resistance would be lost, as may happen with houseflies, if *fatigans* were not exposed to BHC for some time. As the colonies (Penang G and Kuala Lumpur strains) had been maintained separately in the laboratory without exposure to insecticides, it was decided to retest them against BHC; there was unfortunately no time to include dieldrin. As will be seen from the table (1954: Penang G, Kuala Lumpur), it appears that Penang G had in fact lost much of its acquired resistance to BHC, although the MLC was still about twice that of the Kuala Lumpur strain—0.046 p.p.m., as compared with 0.024. Both strains had passed through some ten generations during the interval between the first and the second set of tests.

### Discussion

There are several points which deserve a brief mention.

The MLC's for all three insecticides for the Kuala Lumpur strain of *fatigans* were about twice those found by Wharton<sup>15</sup> for his strain from Tampin, 75 miles (about 120 km) south of Kuala Lumpur:

	BHC	Dieldrin	DDT
Kuala Lumpur	0.025	0.006	0.224
Tampin	0.014	0.003	0.145

This may be due merely to small differences in the testing conditions, or to the possibility that the Kuala Lumpur larvae were slightly older or more vigorous at the time of testing. On the other hand, the Penang B.P. strain, tested at the same time as the Kuala Lumpur strain, had an MLC for BHC (0.017 p.p.m.) similar to that of the Tampin strain (0.014 p.p.m.). Also, in prolonged experiments with the same insecticides in window-trap huts against adult mosquitos, *C. fatigans* at Tampin consistently suffered rather higher mortalities than *fatigans* on the Selangor coast, some 30 miles (48 km) from Kuala Lumpur. Thus, apart from differences due to resistance acquired by exposure to insecticides, it looks as if there may perhaps be local strains of *fatigans* differing somewhat in their initial susceptibility to insecticides.

There are a number of reports of acquired resistance in the *C. pipiens fatigans* group, though only a few, such as the three mentioned below, are based on comparison with a non-resistant strain. Mosna<sup>10</sup> found that adult *C. pipiens autogenicus* in Italy had become resistant to DDT by the beginning of the second season's spraying; this was the first report of resistance in mosquitos. Pal et al.<sup>11</sup> in India tested adult *fatigans* from a village where DDT spraying had been in progress for six years and found them resistant. Hamon<sup>5</sup> reports that on La Réunion resistance develops in *fatigans* about a year after the use of DDT is begun, and he found larvae

resistant to both DDT and BHC; adults were also said to be resistant to DDT, but it seems that they were not compared with a non-resistant strain. Since the MLC of DDT for adult *fatigans* which have never before been exposed to DDT can be so high that it is very difficult to kill them (Wharton <sup>15</sup>), failure to kill *fatigans* with DDT is not by itself proof of acquired resistance.

It seems, therefore, that *C. pipiens* and *C. fatigans* may develop resistance to DDT in as little as one year, thus rivalling houseflies. This compares with five or six years for the salt-marsh mosquitos *Aedes taeniorhynchus* and *sollicitans* in Florida (Deonier & Gilbert;<sup>3</sup> Hess <sup>7</sup>). The BHC resistance of *fatigans* recorded here was first noticed after about two years. There are a number of well-documented reports, especially from Greece (Livadas & Georgopoulos;<sup>9</sup> Belios <sup>1</sup>) of resistance in *Anopheles*, but there is still perhaps some doubt whether this is due to true physiological resistance, or to resistance of a behaviouristic nature. Trapido, who found strong evidence in the field that *A. albimanus* had developed some kind of resistance after from five to eight years of DDT house-spraying,<sup>13</sup> was able to compare the "resistant" strain with a known non-resistant strain that had never been exposed to DDT, and found no difference.<sup>14</sup> Whatever the nature of this resistance in *Anopheles*, it seems to take five or more years to develop.

How strong a resistance can be developed by *pipiens* and *fatigans* is not known. The Georgetown strain in the larval stage (adults were not tested) had acquired a tenfold resistance to BHC, but deliberate exposure and selection in the laboratory might raise this much higher. Resistance to DDT in the salt-marsh *A. taeniorhynchus* and *sollicitans* is said to be about tenfold (Knippling <sup>8</sup>), and in *A. nigromaculis* threefold (Harrison <sup>6</sup>). The *pipiens/fatigans* groups are easily colonized and might offer a suitable alternative to the housefly for resistance studies. Admittedly the genetics of the groups may be complex, but resistance studies would be an added stimulus to the studies on genetics and other aspects of the biology now in progress for taxonomic reasons (Rozeboom <sup>12</sup>).

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### RÉSUMÉ

Après deux ans d'utilisation de l'hexachlorocyclohexane (HCH) comme larvicide à Georgetown, île de Penang, Malaisie, les larves de *Culex fatigans* parurent échapper

à l'action de cet insecticide. On étudia en laboratoire deux colonies de cette espèce de moustique. L'une provenait des canaux de Georgetown où le larvicide ne produisait plus l'effet cherché et l'autre, servant de témoin, de Kuala Lumpur, où aucun insecticide n'avait été utilisé. On détermina la dose létale médiane de HCH, de dieldrine et de DDT pour les deux souches en question. Exposées au HCH, les larves de Georgetown présentèrent une résistance décuple de celle de la souche de Kuala Lumpur, supportant une quantité d'insecticide de 0,257 parties pour un million (0,026 parties chez les témoins). Cette résistance se manifesta également vis-à-vis de la dieldrine, bien que les moustiques n'y aient pas été exposés antérieurement. La résistance au DDT, en revanche, n'était pas accrue. Ces résultats confirment des observations antérieures montrant qu'il existe une résistance croisée entre HCH et dieldrine, qui n'est que très faible entre ces deux insecticides et le DDT.

Les essais furent répétés, un an plus tard, sur la descendance des souches initiales, élevées en laboratoire sans contact avec les insecticides. Au bout de dix générations la résistance initiale de la souche de Georgetown s'était beaucoup affaiblie et n'était plus que le double de celle des témoins.

On a signalé à plusieurs reprises la résistance aux insecticides des *Culex* du groupe *pipiens fatigans*. Il semble que, chez les *Culex* comme chez les mouches domestiques la résistance s'observe au bout d'une année déjà après le début de l'application d'insecticides, tandis qu'elle n'apparaît qu'après 5-6 ans chez les *Anopheles* et les *Aedes* étudiés jusqu'à maintenant.

Il est possible que les moustiques du genre *Culex*, qu'il est facile d'élever en laboratoire puissent, au même titre que les mouches, servir de matériel d'expérience dans l'étude de la résistance aux insecticides.

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